Award Number: W81XWH-12-1-0364

TITLE: Disruption of Calcium Homeostasis during Exercise as a Mediator of Bone Metabolism

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REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 Sept 2014 – 29 Sept 2015

4. TITLE AND SUBTITLE

Disruption of Calcium Homeostasis during Exercise as a Mediator of Bone Metabolism

5a. CONTRACT NUMBER
W81XWH-12-1-0364

5b. GRANT NUMBER
PR110436

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Experiment 2 is completed, and all batched analysis of samples is finished. Regulatory approvals for EXP3 Pilot and EXP3 were completed in Q3/4. Completion of 2 successful infusion pilots and 2 successful oral pilots (and 1 unsuccessful) were completed in Q2-4. Approval of the Continuing Review application, which included local approval to proceed with EXP3, was received 8 May 2015. A further amendment to correct recruitment numbers was approved on 14 August, 2015. Isotopes for the protocol were ordered; delivery occurred 24 August 2015. The isotope has been tested and compounded by the Investigational Pharmacy, and was in use for the most recent pilot infusion experiments. We expect EXP3 to begin in Y4Q1. Wendy Kohrt, PhD, presented preliminary findings from EXP1 and EXP2 at the 2015 Annual Meeting of the American College of Sports Medicine and Sarah Wherry, PhD, has submitted the results from EXP2 for the 2016 Annual Meeting of the American College of Sports Medicine (Appendix A).

15. SUBJECT TERMS
calcium homeostasis, exercise, bone resorption, parathyroid hormone

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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TABLE OF CONTENTS

INTRODUCTION

The **Global Aim** of the proposed research is to investigate a novel mechanism for exercise-related bone loss. We postulate that the disruption of calcium (Ca) homeostasis during acute exercise is a trigger for the activation of bone resorption. The working model portends that excessive dermal Ca loss (i.e., sweating) causes a decline in serum ionized Ca (iCa; the unbound fraction) and triggers an acute increase in parathyroid hormone (PTH). PTH can defend serum Ca by reducing urinary Ca excretion, increasing intestinal Ca absorption, and increasing mobilization of skeletal Ca (bone resorption). If an increase in bone resorption occurs repeatedly over multiple exercise sessions (i.e., exercise training) and is not accompanied by appropriate loading forces to stimulate bone formation, we postulate that this could lead to a decrease in BMD over time. The **Specific Aims** are to **1)** determine whether the magnitude of dermal Ca loss during exercise is a determinant of the decline in iCa and increases in PTH and carboxy-terminal collagen crosslinks (CTX; marker of bone resorption); **2)** determine whether preventing the decline in serum iCa during exercise via intravenous Ca administration (i.e., iCa clamp) prevents an increase in serum PTH and CTX; and **3)** measure serum Ca flux and rate of Ca appearance during exercise and determine whether oral Ca loading before exercise attenuates the increase in serum CTX.

BODY

The following **major tasks** were proposed for Year 3:

- **Complete EXP2**

  Testing for EXP2 was completed on 12 February 2015. We met the goal of having at least 8 men complete EXP2.

  Final Recruitment for EXP2:

	Enrolled	Withdrew	Completed
Men	14	3	11

  Reasons for withdrawals:

  Lack of time (2), non-study-related injury (1)

- **Continue batched assays of serum and sweat samples for EXP2**

  Sample analysis for all participants was completed in February 2015.

- **Hold medical monitor meeting in Q1**

  This meeting was held 19 November 2014.

- **Quarterly data quality assurance evaluations**

  The quarterly data quality evaluations for EXP2 began in April 2015 after being on hold since October 2014. The data manager for the PI's research group resigned her position. Data quality assurance evaluations are up to date.

- **Conduct EXP3 Pilot Visits**

  The University of Colorado Hospital (UCH) Research Pharmacy compounded the first batch of intravenous infusate on 30 December 2014. The second batch was compounded by the Pharmacy on 16 August 2015.

  Two Pilot A (infusion) experiments have been completed. The goal of reaching a steady-state level of isotopic enrichment in 4 hours was achieved.

  Three Pilot C (oral) experiments have been finished. The goal of reaching detectable levels of isotopic enrichment was achieved.

  Progress on EXP3 Pilot A (infusion):

	Enrolled	Withdrew	Completed	Active
Men	3	1	2	0

Reason for withdrawal:

Non-study-related injury/illness (1)

Progress on EXP3 Pilot C (oral):

	Enrolled	Withdrew	Completed	Active
Men	3	0	3	0

- **Begin EXP3**
  Due to delays by our isotope supplier, the study was effectively on hold throughout Summer 2015. Because of the delay in receiving and compounding our infusion isotope, we were not able to complete the Pilot A infusion experiments within the anticipated timeframe. We received COMIRB approval on 14 Aug 2015 to proceed with EXP3, before the second successful pilot infusion experiment was performed, with the acknowledgement that the infusion protocol may require minor modification. No modification will not be necessary because the second pilot infusion experiment was successful.

  We are currently recruiting and screening volunteers for EXP3.

- **Prepare and submit manuscripts**

  Two manuscripts from EXP1 have been prepared but have not yet been submitted. We were invited to submit a brief review on our work on the disruption of calcium homeostasis during exercise for *Exercise and Sport Science Reviews*. We do not want to publish the manuscripts in advance of the review being accepted for publication. Therefore, the manuscripts will be submitted as soon as the review is in press.

  Based on local feedback on the primary manuscript for EXP1, it was suggested that the results of EXP2 may inform the interpretation of EXP1 data. We plan to submit the primary EXP2 manuscript ahead of the EXP1 manuscripts. The EXP2 primary manuscript is currently in process.

  The results from EXP2 will be submitted to the American College of Sports Medicine Annual Meeting 2016. A copy of the submitted abstract is included in Appendix A.

Minor amendments to the protocol have been approved by the local IRB:

PAM011-1:

Tammie Nakamura was removed from the protocol.

PAM012-2:

Enrollment errors (sample size changed to 24 from 14 for EXP3) were corrected; received approval to proceed with EXP3 with infusion rate of 0.417 mg 42Ca/hour; Toby Wellington's role was updated to Primary Contact; Anusha Guntupalli was added to the protocol; Vanessa Sherk was removed from the protocol.

KEY RESEARCH ACCOMPLISHMENTS

The analyses to date do support the hypothesis of EXP2 that preventing the decline in serum ionized calcium at the onset of exercise does prevent increases in PTH and CTX. Preliminary data from EXP1 and EXP2 were presented by Dr. Kohrt in a symposium at the annual meeting of the American College of Sports Medicine in May 2015. She has also been invited to present on the topic of *Disruption of Calcium Homeostasis During Exercise* at the International Sport and Exercise Nutrition Conference in Newcastle, England, in December 2015. Based on feedback from presentations, the research being conducted under this award is viewed as being of high merit and providing novel insights into the disruption of Ca homeostasis during exercise.

REPORTABLE OUTCOMES

Reportable outcomes will be available after the first manuscript undergoes review.

CONCLUSION

No conclusions can yet be drawn.

REFERENCES

The PI is not aware of any published studies that inform this research beyond those included in the grant application.

SUPPORTING DATA: EXPERIMENT 2

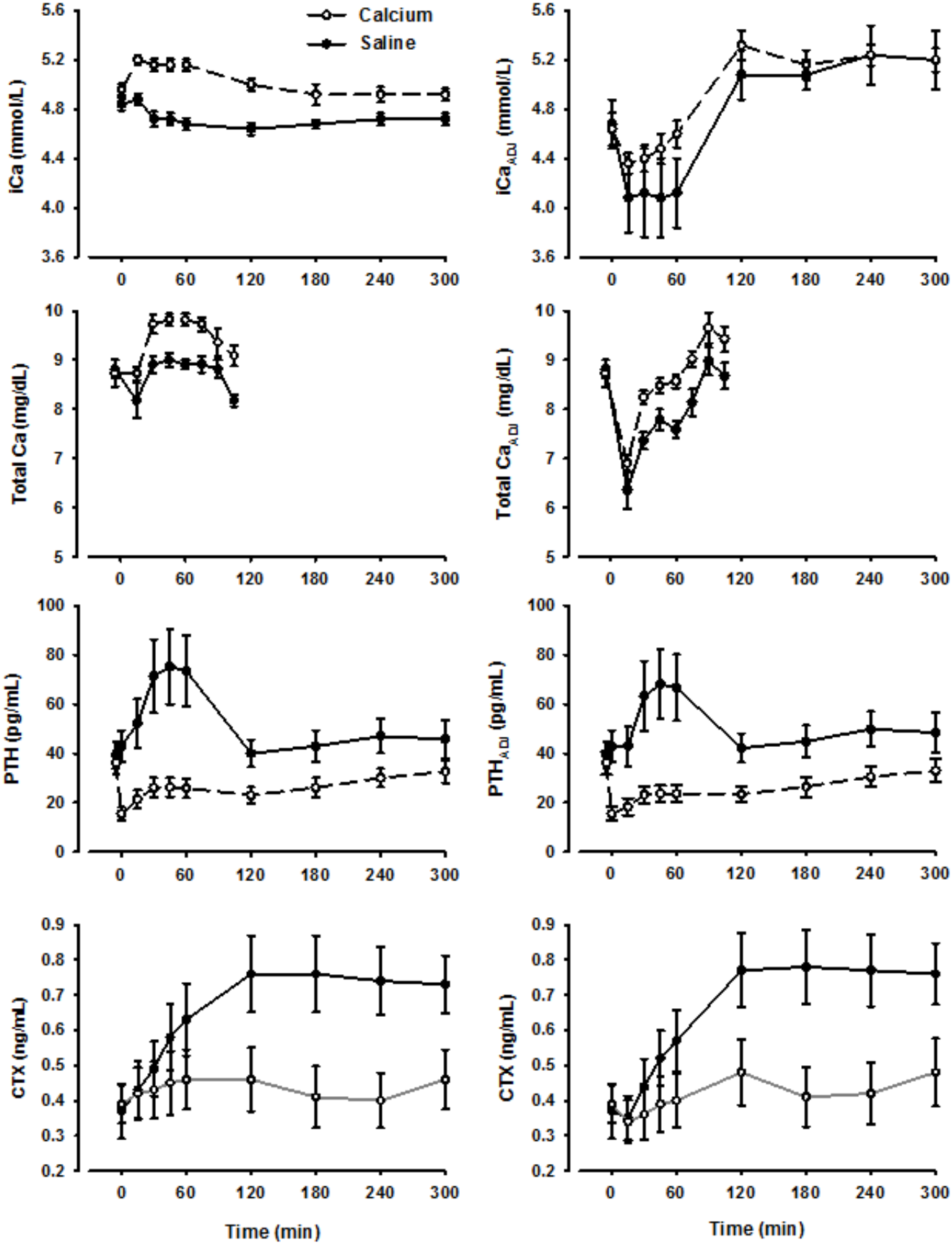
As planned, 11 men completed the study (goal was to have at least 8 finishers out of 14 recruited). The characteristics of the participants are in the following table.

	All (N=11) mean±SD
Age (y)	34.4 ± 5.1
Height (m)	1.78 ± 0.08
Weight (kg)	74.1 ± 9.3
Lumbar spine T-score	0.5 ± 2.4
Total hip T-score	0.5 ± 2.4
Femoral neck T-score	0.1 ± 2.2
Serum calcium (mg/dL)	9.4 ± 0.3
VO_{2peak} (mL/min/kg)	52.9 ± 8.9

All participants completed two exercise bouts, under continuous calcium infusion and continuous saline infusion conditions. The calcium infusion was intended to prevent the exercise-induced decline in serum ionized calcium. We were interested if preventing the calcium decline impacted markers of bone resorption. As indicated in the following table, which shows the change in each marker from before to after the 60 minutes of exercise, the calcium infusion successfully prevented a decline in serum ionized calcium and attenuated the increases in PTH and CTX (adj = adjusted for hemoconcentration; p values reflect the between-group differences in changes).

	Calcium 0-60 min	Saline 0-60 min	p value
iCa (mmol/L)	0.07 ± 0.04	-0.04 ± 0.03	<0.001
iCa_{adj} (mmol/L)	-0.06 ± 0.06	-0.18 ± 0.05	<0.001
Total Ca (mg/dL)	1.09 ± 0.83	0.09 ± 0.54	0.004
Total Ca_{adj} (mg/dL)	-0.16 ± 1.10	-1.23 ± 0.59	0.001
PTH (pg/mL)	-10.4 ± 10.8	34.1 ± 41.5	0.003
PTH_{adj} (pg/mL)	2.2 ± 41.4*	27.4 ± 39.4	0.004
CTX (ng/mL)	0.09 ± 0.14	0.27 ± 0.17	0.001
CTX_{adj} (ng/mL)	0.03 ± 0.12	0.21 ± 0.17	0.003

The following figures illustrate the changes in serum total Ca, iCa, P1NP, PTH, and CTX. The left panels are unadjusted values and the right panels are adjusted for plasma volume shifts. The figures include both exercise (0 to 60 minutes) and the 4-hour recovery (60 to 300 minutes).



APPENDICES

APPENDIX A

Abstract from American College of Sports Medicine Annual Meeting

Maintenance of Serum Ionized Calcium during Exercise Attenuates Exercise-Related Increases in Markers of Bone Resorption

Wherry, S.J., Sherk, V., Wolfe, P., Wellington, T., Quick, J., Boxer, R.S., & Kohrt, W.M.

Exercise is recommended to build or maintain bone mass, but there is evidence that vigorous or prolonged exercise is associated with bone loss under certain conditions. It is our contention that disruptions in calcium homeostasis during exercise lead to increases in parathyroid hormone (PTH) and bone resorption. **PURPOSE:** To determine if preventing the decline in serum ionized calcium (iCa) during exercise attenuates the increases in PTH and a marker of bone resorption (carboxy-terminal collagen crosslinks (CTX)). **METHODS:** Healthy, cycling-trained men (n=11, aged 18-45y) underwent two identical one-hour cycling bouts at ~75% VO_2peak under conditions of calcium gluconate versus normal saline infusion. Blood was sampled every 5 minutes to adjust the calcium infusion rate, with a goal to maintain serum iCa at ~0.2 mg/dL above baseline. The same infusion protocol was replicated under the saline condition. Blood samples to assess PTH, CTX, bone formation (procollagen type 1 amino procollagen (P1NP)), and total calcium (tCa) were taken every 15 minutes during exercise and hourly for 4 hours post-exercise. **RESULTS:** Serum iCa was successfully maintained above baseline during Ca infusion and was significantly different from the saline condition during exercise (all time points $p<0.01$). Compared to saline, the Ca infusion markedly reduced the increase in serum PTH during exercise ($p=0.004$) and the suppression of PTH persisted throughout the 4-hour recovery period (all $p<0.01$). Similarly, the increase in CTX during exercise was suppressed with Ca infusion ($p=0.003$) and remained below the saline condition through recovery (all $p<0.001$). tCa was also a significantly higher during exercise ($p<0.001$) during Ca infusion, but conditions were similar during recovery (all $p>0.10$). There were no differences between conditions for P1NP at any time points (all $p>0.10$). **CONCLUSIONS:** The increase in bone resorption was attenuated when the exercise-related decline in serum iCa was prevented, suggesting a calcium-dependent relationship. There was no effect of Ca infusion on P1NP, but the duration of post-exercise sampling may have been too short to capture any changes. The results are limited to young, trained, men during cycling exercise. Future research should investigate sex- and age-differences and other exercise modalities.